

the Patent Office is authorized to charge the underpayment to Deposit Account No. 04-1073.

AMENDMENTS

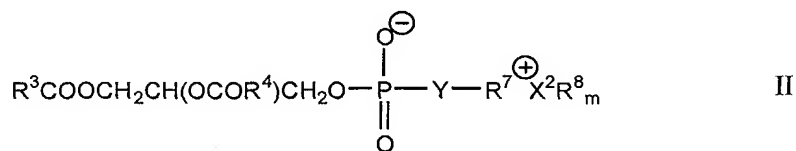
In the Claims:

Please cancel claim 1 without prejudice.

Please add new claims 21-49 pursuant to 37 C.F.R. § 1.121(c)(1)(i) as set forth in the "clean" version set forth below. Entry is respectfully requested.

21(NEW). An oral vaccine comprising a nucleic acid operatively encoding an antigen complexed with or entrapped within liposomes formed from liposome forming components and comprising

- a) at least one cationic compound;
- b) zwitterionic phospholipid consisting of one or two compounds having the general formula II



in which R^3 and R^4 are the same or different and are a group of the formula $\text{CH}_3(\text{CH}_2)_e(\text{CH}=\text{CH}-\text{CH}_2)_f(\text{CH}_2)_g-$ in which f is 0 to 6, each of e and g are 0 to 23 and $e + g$ and $3f$ is in the range 12 to 23;

R^7 is a C_{1-8} alkanediyl group;

Y is $-\text{O}-$ or a bond;

X^2 is N, P or S;

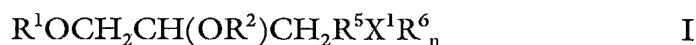
m is 3 when X^2 is N or P and is 2 when X^2 is S; and

the groups R^8 are the same or different and are selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{6-11} aryl or aralkyl, or two or three of the groups R^8 together with X^2 form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms,

in which at least 50% by mole of groups R^3 and R^4 has a value for f of 0 and which comprises a compound in which R^3 and R^4 are the same and have a value for f of 0:

in which the molar ratio of cationic compound to zwitterionic phospholipid is in the range of 1:1 to 1:10.

22(NEW). A vaccine according to claim 21 in which the cationic compound has the general formula I,



in which R^1 and R^2 are the same or different and are a group of the formula $CH_3(CH_2)_a(CH=CH-CH_2)_b(CH_2)_c(CO)_d-$ in which b is 0 to 6, a and c are each selected from 0-23 and $(a + c + 3b)$ is in the range 12-23 and d is 0 or 1;

R^5 is a bond or a C_{1-8} alkanediyl group;

X^1 is N, P or S;

n is 3 where X^1 is N or P and is 2 where X^1 is S; and

the groups R^6 are the same or different and are selected from the group consisting of hydrogen, C_{1-8} alkyl, C_{6-12} aryl and aralkyl, or two or three of the groups R^6 together with X^1 form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms.

23(NEW). A vaccine according to claim 22 in which R^1 is the same as R^2 and R^3 is the same as R^4 .

24(NEW). A vaccine according to claim 23 in which R^1 and R^2 represent a different group to R^3 and R^4 .

25(NEW). A vaccine according to claim 23 in which R^1 and R^2 represent a different group to R^3 and R^4 , and in which in R^1 and R^2 , b is 1, and in which $(a + c)$ is in

the range 10 to 20.

26(NEW). A vaccine according to claim 23 in which d is 0.

27(NEW). A vaccine according to claim 22 in which X^1 is N and in which the R^6 groups are all C_{1-4} alkyl.

28(NEW). A vaccine according to claim 21 which comprises two zwitterionic phospholipids in each of which Y is O, X^2 is N, and the groups R^8 of the first phospholipid are all hydrogen and the groups R^8 of the second phospholipid are all C_{1-4} alkyl.

29(NEW). A vaccine according to claim 28 in which, in each phospholipid R^7 is $(CH_2)_h$ in which h is 2 or 3.

30(NEW). A vaccine according to claim 28 in which the groups R^3 and R^4 of the said first phospholipid are the same and each is a group in which f is 1 and $(e + g)$ is in the range 10 to 20.

31(NEW). A vaccine according to claim 30 in which in the groups R^3 and R^4 of the said second phospholipid are the same, f is O and $e + g$ is in the range 15 to 23.

32(NEW). A vaccine according to claim 31 in which the said second zwitterionic phospholipid is selected from the group consisting of distearoylphosphatidylcholine, distearoylphosphatidylethanolamine, dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine.

33(NEW). A vaccine according to claim 21 in which the cationic compound is cholesterol-3 β -N-(dimethylaminoethyl) carbamate.

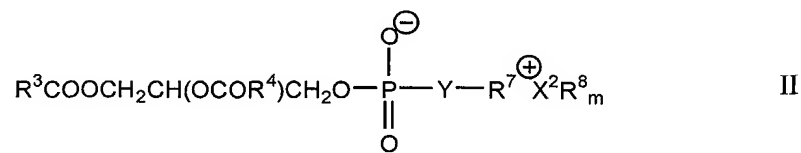
34(NEW). An oral vaccine according to claim 21 in which the liposome forming components include at least 25 mole% of components which individually have a transition temperature of more than 40°C.

35(NEW). A vaccine according to claim 21 in which the nucleic acid is entrapped within the liposomes.

36(NEW). A method of entrapping polynucleotide into liposomes involving the steps of:

- i) forming an aqueous suspension comprising naked nucleic acid, which operatively encodes an immunogenic polypeptide useful to induce a desired immune response in a human or animal subject, and preformed liposomes formed of liposome forming components comprising
 - a) at least one cationic compound;
 - b) zwitterionic phospholipid consisting of one or two compounds having the

general formula II



in which R³ and R⁴ are the same or different and are selected from groups of the formula CH₃(CH₂)_e(CH=CH-CH₂)_f(CH₂)_g- in which f is 0 to 6, each of e and g are 0 to 23 and e + g and 3f is in the range 12 to 23;

R⁷ is a C₁₋₈ alkanediyl group;

Y is -O- or a bond;

X² is N, P or S;

m is 3 when X² is N or P and is 2 when X² is S; and

the groups R⁸ are the same or different and are selected from the group consisting of hydrogen, C₁₋₈ alkyl, C₆₋₁₁ aryl or aralkyl, or two or three of the groups R⁸ together with X²

form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms,

in which at least 50% by mole of groups R^3 and R^4 has a value for f of 0 and which comprises a compound in which R^3 and R^4 are the same and have a value for f of 0:

in which the molar ratio of cationic compound to zwitterionic phospholipid is in the range of 1:1 to 1:10.

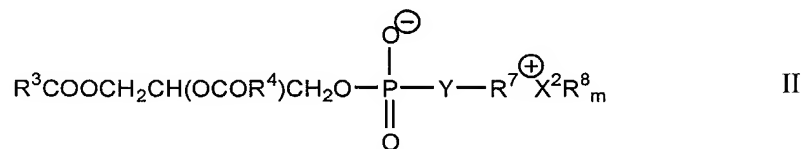
- ii) freeze-drying or spray-drying the suspension, and
- iii) rehydrating the product of step ii) to form dehydration/rehydration vesicles.

37(NEW). A method according to claim 36 comprising the further steps of:

- iv) subjecting the aqueous suspension of dehydration/rehydration vesicles from step iii to microfluidization to control their size; and
- v) optionally separating non-entrapped nucleic acid from liposomes.

38(NEW). Method of vaccinating an animal comprising administering orally a composition comprising a nucleic acid operatively encoding an antigen complexed with or entrapped within liposomes formed from liposome forming components comprising

- a) at least one cationic compound
- b) zwitterionic phospholipid consisting of one or two compounds having the general formula II



in which R^3 and R^4 are the same or different and are a group of the formula $CH_3(CH_2)_e(CH=CH-CH_2)_f$ in which f is 0 to 6, each of e and $g + 3f$ are 0 to 23 and $e + g$ is in the range 12 to 23;

R^7 is a C_{1-8} alkanediyl group;

Y is -O- or a bond;

X² is N, P or S;

m is 3 when X² is N or P and is 2 when X² is S; and

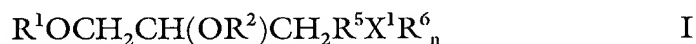
the groups R⁸ are the same or different and are selected from the group consisting of hydrogen, C₁₋₈ alkyl, C₆₋₁₁ aryl or aralkyl, or two or three of the groups R⁸ together with X² form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms;

in which at least 50% by mole of groups R³ and R⁴ has a value for f of 0 and which comprises a compound in which R³ and R⁴ are the same and have a value for f of 0,

wherein the molar ratio of cationic compound to zwitterionic phospholipid is in the range 1:1 to 1:10,

whereby an immune response to the said antigen is generated.

40(NEW). A method according to claim 38 in which the cationic compound has the general formula I,



in which R¹ and R² are the same or different and are a group of the formula CH₃(CH₂)_a(CH=CH-CH₂)_b(CH₂)_c(CO)_d- in which b is 0 to 6, a and c are each selected from 0-23 and (a + c + 3b) is in the range 12-23 and d is 0 or 1;

R⁵ is a bond or a C₁₋₈ alkanediyl group;

X¹ is N, P or S;

n is 3 where X¹ is N or P and is 2 where X¹ is S; and

the groups R⁶ are the same or different and are selected from the group consisting of hydrogen, C₁₋₈ alkyl, C₆₋₁₂ aryl and aralkyl, or two or three of the groups R⁶ together with X¹ form a saturated or unsaturated heterocyclic group having 5 to 7 ring atoms.

41(NEW). A method according to claim 40 in which R¹ is the same as R² and R³ is the same as R⁴.

42(NEW). A method according to claim 41 in which R¹ and R² represent a

different group to R^3 and R^4 .

43(NEW). A method according to claim 41 in which R^1 and R^2 represent a different group to R^3 and R^4 , in which in R^1 and R^2 , b is 1, and in which $(a + c)$ is in the range 10 to 20.

44(NEW). A method according to claim 38 in which the liposome forming materials comprise two zwitterionic phospholipids in each of which Y is O, X^2 is N, and the groups R^8 of the first phospholipid are all hydrogen and the groups R^8 of the second phospholipid are all C_{1-14} alkyl, and R^7 is $(CH_2)_h$ in which h is 2 or 3.

45(NEW). A method according to claim 44 in which the groups R^3 and R^4 of the said first phospholipid are the same and each is a group in which f is 1 and $(e + g)$ is in the range 10 to 20.

46(NEW). A method according to claim 45 in which in the groups R^3 and R^4 of the said second phospholipid are the same f is 0 and $e + g$ is in the range 15 to 23.

47(NEW). A method according to claim 46 in which the said second zwitterionic phospholipid is selected from the group consisting of distearoylphosphatidylcholine, distearoylphosphatidylethanolamine, dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine.

48(NEW). A method according to claim 38 in which the cationic compound is cholesterol-3 β -N-(dimethylaminoethyl) carbamate.

49(NEW). A method according to claim 38 in which the nucleic acid is entrapped within the liposomes.